ANTICANCER RESEARCH *37*: 6815-6824 (2017)
doi:10.21873/anticanres.12142

Adjuvant Breast Cancer Treatments Induce Changes in Homoarginine Level – A Prospective Observational Study

HANNA AULA[1,2], TANJA SKYTTÄ[1,2], SUVI TUOHINEN[1,3,4], TIINA LUUKKAALA[5], MARI HÄMÄLÄINEN[6], VESA VIRTANEN[1,3], PEKKA RAATIKAINEN[4], EEVA MOILANEN[6] and PIRKKO-LIISA KELLOKUMPU-LEHTINEN[1,2]

[1]*Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland ;*
[2]*Department of Oncology, Tampere University Hospital, Tampere, Finland;*
[3]*Heart Hospital, Tampere University Hospital, Tampere, Finland;*
[4]*Department of Cardiology, Heart and Lung Center, Helsinki University Hospital, Helsinki, Finland;*
[5]*Science Center, Pirkanmaa Hospital District, University of Tampere, Tampere, Finland;*
[6]*The Immunopharmacology Research Group, Faculty of Medicine and Life Sciences, University of Tampere and Tampere University Hospital, Tampere, Finland*

Abstract. *Aim: To identify patients with breast cancer at risk for cardiotoxicity, we evaluated homoarginine (HA) behavior during adjuvant treatment. Patients and Methods: Eighty-one patients received radiotherapy (RT) with or without endocrine treatment, and 19 received chemotherapy, RT and endocrine therapy. Serum HA, asymmetric dimethylarginine (ADMA) and high-sensitivity cardiac troponin T (hscTnT) were measured and echocardiography was performed before chemotherapy, and before and after RT. Results: In chemo-naïve tamoxifen users HA increased during RT from a median (IQR) of 2.47 (1.61-3.35) to 2.86 (1.93-4.23) μM (p=0.028) and remained stable in patients with aromatase inhibitor and in those without endocrine therapy. Tamoxifen users were mostly spared from echocardiographic changes. In chemotherapy-treated patients, HA decreased during chemotherapy (p=0.001) from 1.46 (1.01-2.18) to 0.91 (0.71-1.29) μM, and increased (p=0.004) to 1.19 (0.83-1.63) μM during RT, remaining lower than at baseline (p=0.014). Echocardiographic changes were observed during chemotherapy. Conclusion: HA decrease during chemotherapy could indicate an increased risk of cardiovascular morbidity. Additionally, HA increase in tamoxifen users may reflect a cardioprotective effect of tamoxifen.*

Correspondence to: Hanna Aula, MD, Department of Oncology, Tampere University Hospital, PO Box 2000, 33521 Tampere, Finland. Tel: +358 331163118, Mob: +358 503863794, Fax: +358 331163001, e-mail: hanna.aula@uta.fi

Key Words: Breast cancer, cardiotoxicity, homoarginine, adjuvant treatments.

In order to lower the risk of recurrence and death from early breast cancer, patients receive adjuvant treatments. However, cardiotoxicity, manifesting years later, is associated with all forms of adjuvant treatment: chemotherapy, radiotherapy (RT) and endocrine therapy (1-6). Furthermore, a recent meta-analysis concluded that the 19% increase in risk of cardiovascular events with aromatase inhibitor (AI) use relative to tamoxifen was most likely a reflection of the cardioprotective effect of tamoxifen (7).

Low levels of the cardiac biomarker, homoarginine (HA), have been associated with an increased risk of cardiovascular events, and increased cardiac and all-cause mortality, in patients with various heart conditions in population-based studies (8-14). An association of low HA with impaired cardiac function has also been documented, namely, with a lowered left ventricular ejection fraction (LVEF) and diastolic dysfunction in patients with preserved LVEF (8, 15). In contrast, an elevated level of another cardiac biomarker, asymmetric dimethylarginine (ADMA), is associated with increased cardiovascular risk (16). Although an epidemiological study found no association between HA and cancer-specific mortality, the effect of cancer treatments on HA level has not been reported to our knowledge (12).

The aim of our study was to investigate changes in cardiac biomarkers, HA, ADMA and high-sensitivity cardiac troponin-T (hscTnT), and in echocardiographic measurements during and after adjuvant therapy of early breast cancer to find patients at risk for later cardiovascular complications.

Patients and Methods

Patient population. This single-center, prospective, observational clinical study included 100 patients with measurable serum samples

who received adjuvant treatment for breast cancer or ductal carcinoma *in situ* between June 2011 and May 2013. All patients received postoperative RT after breast-conserving surgery or mastectomy. Patients were grouped according to chemotherapy and endocrine therapy (Figure 1). Exclusion criteria were described previously, with the exception of the inclusion of chemotherapy-receiving patients in this study (17). The protocol was approved by the local Ethics Committee (R10160) and informed consent was obtained from all individual participants included in the study.

Chemotherapy. The most common chemotherapy regimen used was three courses of docetaxel (80 mg/m^2), followed by three courses of CEF (600 mg/m^2 fluorouracil, 75 mg/m^2 epirubicin and 600 mg/m^2 cyclophosphamide; n=20). Other regimens used were six courses of CEF (n=4); four courses of docetaxel (75 mg/m^2) and cyclophosphamide (600 mg/m^2) (n=1); or three courses of docetaxel (60 mg/m^2) and capecitabine (900 mg/m^2 twice daily on days 1-15) followed by three courses of cyclophosphamide (600 mg/m^2), epirubicin (75 mg/m^2) and capecitabine (900 mg/m^2 twice daily on days 1-15) (n=3). All cytotoxic agents, except capecitabine, were administered intravenously on day one of the 21-day treatment cycle (18-20). One patient also received trastuzumab (8 mg/kg loading dose and 6 mg/kg thereafter) together with docetaxel.

Radiotherapy. Execution of the RT was described in detail in earlier publications (17, 21). The treatment dose was either 50 Gy in 2-Gy fractions with or without an additional boost of 10-16 Gy in 2-Gy fractions to the tumor bed or 42.56 Gy in 2.66-Gy fractions over 3.5 weeks (hypofractionation). Axillary or supraclavicular lymph nodes were included in the planning target volume according to local guidelines.

Serum biomarker analysis. Serum samples were taken before commencing chemotherapy, if applicable, before RT, 2 weeks (for hypofractionated RT) or 3 weeks (for conventional RT) after the start of RT and the last day of RT. Serum samples were subjected to solid-phase extraction (Oasis® MCX SPE columns; Waters, Milford, MA, USA) as described previously (22). L-NG-Monomethyl arginine was used as internal standard. Eluted samples were dried under nitrogen at 55˚C and the residues were dissolved in 0.1 mL ultrapure water and stored at –20˚C. Chromatography was performed on a Symmetry® C18 column (4.6×150 mm, 5 μm) with Symmetry® C18 guard column (3.9×20 mm) (Waters) as described previously (23) using a Shimadzu liquid chromatography system (Shimadzu, Kyoto, Japan) with a gradient pump (LC-10AD), an autosampler (SIL-30AC) and fluorescence detector (RF-10Axl). Data acquisition and analysis were performed using LabSolutions software (Shimadzu), and ADMA and HA concentrations in samples were calculated against standard curves. The detection limits and inter-assay coefficients of variation for AMDA were 0.078 μM and 4.9%, respectively, and 0.078 μM and 1.7% for HA.

High sensitivity cardiac troponin T was measured at the same time points as HA and ADMA. The detection limit was 5 ng/l and values below this were estimated to be 4 ng/l.

Echocardiographic examination. Echocardiographic examination was performed before the beginning of chemotherapy, before the start of RT and after completion of RT by a single cardiologist (ST) using a commercially available ultrasound machine (Philips iE33 ultrasound system; Philips, Bothell, WA, USA) and a 1-5 MHz matrix-array X5-1 transducer in a standardized manner as described previously (24), following current guidelines (25-28).

Statistical analysis. Chemo-naïve patients and chemotherapy-treated patients were analyzed separately. Furthermore, patients were divided into subgroups by endocrine therapy. Due to the skewed distributions of all continuous variables, medians and interquartile ranges were calculated. To test differences in baseline characteristics, Fisher's exact test and the Mann–Whitney *U*-test were used for catecorical and continuous variables, respectively. Linear regression was used to determine statin use as a predictor of change in HA during RT in chemo-naïve patients. In order to test for changes in HA, ADMA, hscTnT and echocardiographic parameters during chemotherapy and RT, the Friedman and Wilcoxon signed-rank tests were utilized. Group based trajectory modeling was used to created trajectory groups of chemo-naïve patients for HA (29). The trajectory groups were created according to all measurements of HA in each patient as a continuous outcome measure and the groups represent clusters of individuals with similar trajectories and outcomes over time (30). Models were fitted by using the flexmix package (31) of the statistical program R, version 3.3.0, from the R Foundation for Statistical Computing (32). Relative goodness of fit was assessed using Bayesian information criteria (BIC). Odds ratio (OR) for the trajectory groups was determined by multinomial logistic regression. Statistical analyses were performed using IBM SPSS statistics for Windows (version 23, IBM Corp., Armonk, NY, USA). *p*-Values under 0.05 were considered statistically significant.

Results

Baseline characteristics. Baseline characteristics of all treated patients and groups according to oncological intervention are described in Table I. Patients receiving chemotherapy were younger (p=0.002) and more likely to receive tamoxifen (p=0.010) or AI (p=0.019) than chemo-naïve patients. However, baseline HA levels were similar (p=0.772). The baseline ADMA level was significantly higher (p<0.001) in chemo-naïve patients than in chemotherapy-receiving patients, albeit the absolute levels were similar, at 0.40 and 0.39, respectively.

Patients without endocrine therapy had a lower body mass index (BMI) than tamoxifen users (p=0.033) or AI users (p=0.003) (Table II). Tamoxifen users used angiotensin-converting enzyme inhibitors more often than those without endocrine therapy (p=0.031).The baseline HA level was significantly higher in tamoxifen users than in AI users (p=0.029) and those without endocrine therapy (p=0.016). The ADMA level, on the other hand, tended to be lower in tamoxifen users than in AI users (p=0.076) and those without endocrine therapy (p=0.054).

Homoarginine changes during RT in chemo-naïve patients. HA remained stable in the 81 patients who received RT but no chemotherapy, 1.58 (1.12-2.035) μM before RT and 1.60 (0.98-2.035) μM after RT (p=0.822) (Figure 2a). Analysis was also performed according to tamoxifen use. As baseline

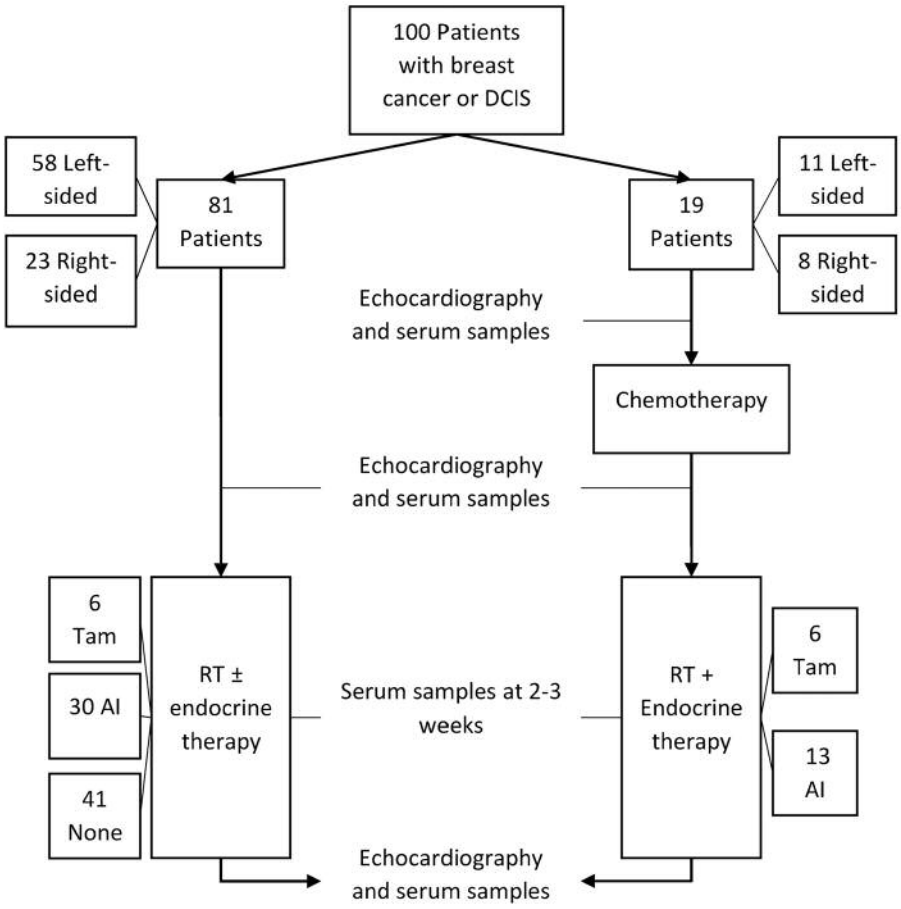

Figure 1. *Flow chart of patients included in the study. DCIS: Ductal carcinoma in situ, RT: radiotherapy, AI: aromatase inhibitor, Tam: tamoxifen.*

Table I. *Baseline characteristics of the study population.*

Characteristic	All treated (n=100)	Chemo + RT (n=19)	RT only (n=81)	*p*-Value*
Median age (IQR), years	62 (58-66)	58 (50-61)	64 (58-66)	0.002
Median BMI (IQR), kg/m2	25.95 (24.10-28.95)	24.90 (23.10-28.15)	26.20 (24.21-29.31)	0.195
Current smoker, n (%)	14 (14)	4 (21.1)	10 (12.3)	0.460
Statin use, n (%)	18 (18)	1 (5.3)	17 (21)	0.183
Hypertension, n (%)	37 (37)	5 (26.3)	32 (39.5)	0.429
CAD, n (%)	4 (4)	1 (5.3)	3 (3.7)	0.576
ACE or ARB, n (%)	27 (27)	3 (15.8)	24 (29.6)	0.265
Hypothyreosis, n (%)	15 (15)	3 (15.8)	12 (14.8)	1.000
ASA, n (%)	8 (8)	0 (0)	8 (9.9)	0.347
Beta-blockers, n (%)	15 (15)	3 (15.8)	12 (14.8)	1.000
AI, n (%)	43 (43)	13 (68.4)	30 (37)	0.019
Tamoxifen, n (%)	12 (12)	6 (31.6)	6 (7.4)	0.010
Median HA (IQR), µM	1.56 (1.10-2.08)	1.46 (1.01-2.18)	1.58 (1.12-2.035)	0.772
Median ADMA (IQR), µM	0.39 (0.3-0.42)	0.39 (0.33-0.44)	0.40 (0.37-0.44)	<0.001
Median hscTnT (IQR), ng/l,	4.0 (4.0-6.0)	4.0 (4.0-4.0)	4.0 (4.0-6.75)	0.181

Chemo: Chemotherapy, RT: radiotherapy, IQR: interquartile range, BMI: body mass index, CAD: coronary artery disease, ACE: angiotensin-converting enzyme inhibitors, ARB: angiotensin II receptor blockers, ASA: low-dose acetylsalicylic acid, AI: aromatase inhibitor use, HA: homoarginine, ADMA: asymmetric dimethylarginine, hscTnT: high-sensitivity cardiac troponin-T. *Mann–Whitney *U*-test for non-normal variables, Fischer's exact test for categorical variables to compare chemo + RT and radiotherapy-only groups.

Table II. *Baseline characteristics of patients receiving radiotherapy (RT) and tamoxifen, aromatase inhibitor (AI) or no endocrine therapy.*

Characteristic	Radiotherapy			*p*-Value[1]	*p*-Value[2]	*p*-Value[3]
	Tamoxifen (n=6)	AI (n=30)	No endocrine therapy (n=45)			
Median age (IQR), years	64 (56.75-69.25)	64 (58-67)	62 (58-66)	0.951	0.638	0.458
Median BMI, kg/m^2, (IQR)	30.4 (25.7-33.2)	27.4 (24.9-30.3)	24.8 (23.6-27.4)	0.448	0.033	0.003
Current smoker, n (%)	0 (0)	5 (16.7)	5 (11.1)	0.564	1.000	0.508
Statin use, n (%)	2 (33.3)	8 (26.7)	7 (15.6)	1.000	0.284	0.255
hypertension, n (%)	4 (66.7)	13 (43.3)	15 (33.3)	0.391	0.179	0.467
CAD, n (%)	0 (0)	1 (3.3)	2 (4.4)	1.000	1.000	1.000
ACE or ARB, n (%)	4 (66.7)	11 (36.7)	9 (20.0)	0.210	0.031	0.121
Hypothyreosis, n (%)	1 (16.7)	6 (20.0)	5 (11.1)	1.000	0.548	0.330
ASA, n (%)	1 (16.7)	5 (16.7)	2 (4.4)	1.000	0.319	0.108
Beta-blockers, n (%)	1 (16.7)	7 (23.3)	4 (8.9)	1.000	0.480	0.104
Median mean heart dose, (IQR)	1.74 (0.62-4.17)	2.30 (1.06-3.75)	2.02 (0.80-3.68)	0.749	0.831	0.799
Median baseline HA, (IQR), μM	2.47 (1.61-3.35)	1.59 (1.12-1.96)	1.52 (1.09-1.95)	0.029	0.016	0.996
Median baseline ADMA (IQR), μM	0.37 (0.32-0.39)	0.41 (0.37-0.44)	0.40 (0.37-0.45)	0.076	0.054	0.872
Median baseline hscTnT (IQR), ng/l	4.5 (4.0-17.3)	5.0 (4.0-8.0)	4.0 (4.0-6.0)	0.782	0.414	0.157

AI: Aromatase inhibitor use, IQR: interquartile range, BMI: body mass index, CAD: coronary artery disease, ACE: angiotensin-converting enzyme inhibitors, ARB: angiotensin II receptor blockers, ASA: low-dose acetylsalicylic acid, HA: homoarginine, ADMA: asymmetric dimethylarginine, hscTnT: high-sensitivity cardiac troponin-T. [1]Tamoxifen *vs.* AI, [2]tamoxifen *vs.* no endocrine therapy, [3]AI *vs.* no endocrine therapy. Mann–Whitney *U*-test for non-normal variables, Fischer's exact test for categorical variables.

HA values and the change in HA levels were similar in AI users and in those without endocrine therapy, these patients were analyzed together as one group. Tamoxifen users had a higher median HA of 2.47 (1.61-3.35) μM before starting RT compared to the median HA of 1.53 (1.1-1.95) μM in non users (p=0.017). The HA level also increased significantly by 0.47 (0.31-0.89) μM (p=0.028) in tamoxifen users (Figure 2b), whereas the change of −0.03 (−0.25-0.1) μM (p=0.231) in non users was not significant (Figure 2c). In linear regression analysis, statin use was a significant factor predicting the change in HA. Statin use did not affect the baseline HA value, which was 1.50 (0.98-2.17) μM in statin users and 1.60 (1.14-1.98) μM in non users (p=0.826), but there was a significant increase of HA by 0.1 (0.01-0.56) μM (p=0.014) in statin users and a non-significant change of −0.1 (−0.33-0.14) μM (p=0.123) in non users during RT.

The chemo-naive patients were also divided into trajectories by HA behavior (Figure 3). Group 1 included four out of 11 (36%) tamoxifen users and group 2 included two out of 43 (4.7%) tamoxifen users. There were no tamoxifen users among the 27 patients in group 3. The odds ratio for tamoxifen use was 11.71 (95% confidence interval=1.79-76.55) between groups 1 and 3.

Homoarginine changes in patients receiving chemotherapy. The median HA values, in Figure 4, for the 19 patients who received both chemotherapy and RT were 1.46 (1.01-2.18) μM, 0.91 (0.71-1.29) μM and 1.19 (0.83-1.63) μM before chemotherapy, before RT and after RT, respectively. The changes in HA during chemotherapy and RT were significant (p<0.001 Figure 4). HA values decreased by 0.52 μM during chemotherapy (p=0.001) and increased by 0.20 μM during RT (p=0.004), but did not recover to baseline levels. HA levels remained 0.27 (−0.37-0.02) μM lower at the end of RT compared to baseline levels before chemotherapy (p=0.014).

All of these 19 patients received endocrine therapy, six with tamoxifen and 13 with AI. The baseline values and the decrease in HA levels during chemotherapy were similar in tamoxifen and AI users. During the RT following chemotherapy, HA values increased by a median of 0.71 (0.09-1.26) μM (p=0.075) in tamoxifen users and by 0.15 (0.03-0.38) μM (p=0.023) in AI users. HA values at the end of RT were 1.48 (1.14-2.81) μM in tamoxifen users and 0.96 (0.78-1.43) μM in AI users. Although, the difference in end values was only borderline significant, p=0.058, between the groups, HA levels remained significantly lower in AI users (p=0.002) than the baseline HA values. In tamoxifen users, there was no significant difference in HA levels between baseline and after RT (p=0.753).

Asymmetric dimethylarginine (ADMA). In chemo-naïve patients, the median ADMA levels were 0.40 (0.37-0.44) μM and 0.41 (0.35-0.45) μM before and after RT, respectively and they remained stable throughout RT (p=0.569). ADMA levels were compared according to tamoxifen use and the baseline levels tended to be lower (p=0.050) in tamoxifen users (Table

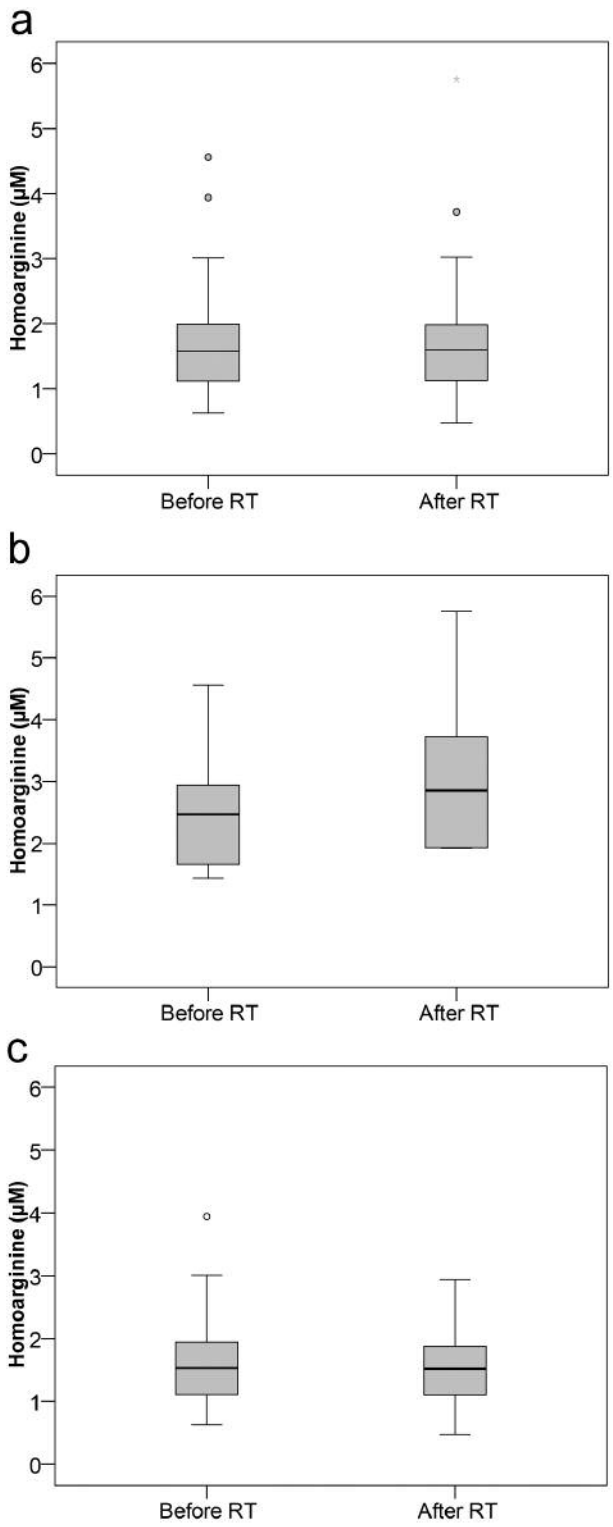

Figure 2. *Homoarginine (HA) levels in patients before and after radiotherapy (RT). The HA level in all chemo-naïve patients remained stable after RT (a). The HA level before RT was higher in tamoxifen users (b) compared to aromatase inhibitor users and those without endocrine therapy (c); while HA increased in tamoxifen users during RT, it remained stable in non-tamoxifen users.*

II) than in non users [0.40 (0.37-0.44) µM]. The difference persisted and became significant at the end of radiotherapy (p=0.044), with ADMA levels of 0.35 (0.31-0.38) µM in tamoxifen users and 0.41 (0.37-0.45) µM in non users.

In patients receiving chemotherapy and RT, the median ADMA levels were 0.39 (0.33-0.44) µM, 0.41 (0.31-0.47) µM and 0.37 (0.32-0.42) µM at baseline, and before RT and after RT, respectively (p=0.215). The median change in ADMA was 0.02 (−0.02-0.05) µM during chemotherapy (p=0.146) and −0.02 (−0.056-0.01) µM during radiotherapy (p=0.076). Neither tamoxifen nor AI use significantly affected levels of or changes in ADMA in chemotherapy-receiving patients.

High-sensitivity cardiac troponin T. We reported radiotherapy-induced changes in hscTnT in chemo-naïve patients with left-sided breast cancer in a previous publication (21). The current study population also included patients with right-sided breast cancer and in this population, hscTnT remained stable, 4 (4-6.75) ng/l before RT and 5 (4-7.5) ng/l after RT (p=0.116). Endocrine therapy did not affect baseline TnT or the change in hscTnT.

In patients receiving chemotherapy, hscTnT increased during chemotherapy from 4 (4-4) ng/l to 9 (7-14) ng/l and continued to increase during RT up to 13 (9-16) ng/l. Endocrine therapy did not affect the hscTnT level, which was similar before chemotherapy, before RT and after RT in tamoxifen and AI users alike. Regardless, during RT, the increase in hscTnT from 13 (8.5-13) ng/l to 14 (8-27.5) ng/l was not significant (p=0.750), whereas in AI users the increase from 9 (7-10.5) ng/l to 12 (9-17) ng/l was significant (p=0.006).

Echocardiographic measurements. The detailed echocardiographic changes in the chemo-naïve study population have been described in previous publications (33, 34). Table III shows a previously unpublished comparison of the echocardiographic changes according to endocrine therapy of patients with measurable serum samples. At baseline, AI users had lower septal cyclic variation of the integrated backscatter (CVIBS) and higher septal integrated backscatter in end-diastole calibrated to the pericardium (cIBS) than tamoxifen users and those without endocrine therapy. The six patients using tamoxifen had the only significant change in CVIBS (p=0.046), whereas AI users had significant changes in tricuspid annular plane systolic excursion (TAPSE), global longitudinal strain in speckle tracking analysis (GLS) and mitral inflow E-wave peak velocity in pulsed Doppler analysis (mitral E) and those without endocrine therapy had significant changes in CVIBS, cIBS, TAPSE, interventricular septum thickness (IVS) and posterior wall (PW) (Table III).

Eleven patients with left-sided breast cancer receiving chemotherapy had an echocardiographic examination

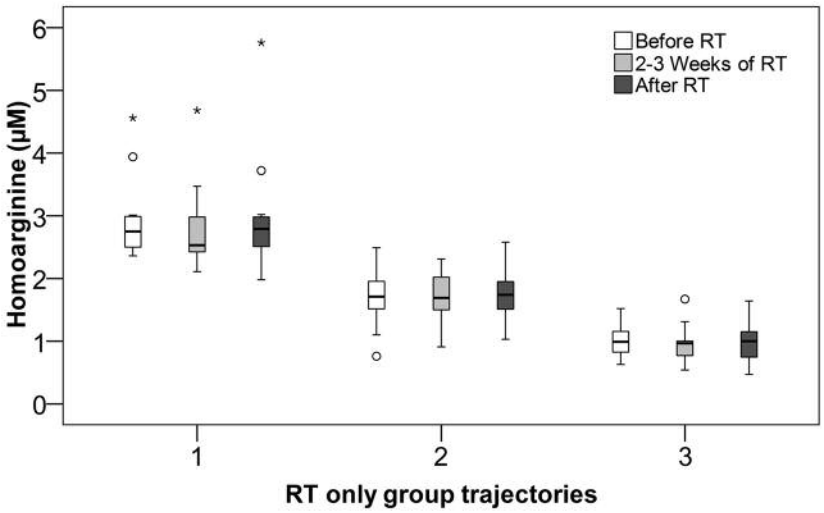

Figure 3. *Homoarginine (HA) behavior in three trajectory groups in chemo-naïve patients. All tamoxifen users were included in groups 1 or 2.*

performed before chemotherapy, before RT and after RT. During chemotherapy, there was a significant increase in tricuspid regurgitation peak gradient (Tr grad) (n=10, p=0.042) from 18.5 (13.3.-21.3) mm to 20.5 (19.0-22.5) mm, an increase in left ventricular end-systolic diameter (LVESD) (n=10, p=0.041) from 31.2 (27.8-32.7) mm to 33.0 (30.7-33.5) mm, an increase in PW (n=10, p=0.032) from 9.0 (8.3-9.6) mm to 9.6 (9.0-11.0) mm, and a decrease in CVIBS (n=10, p=0.037) from 11.92 (9.03-14.30) dB to 9.73 (6.62-11.40) dB. During RT, there were no significant changes in echocardiography in these 11 patients. Although RT did not significantly affect LVESD measurement, LVESD did not return to the baseline level but remained significantly increased at 32.7 (30.7-33.0) mm compared to baseline measurements (p=0.041). Endocrine therapy did not affect echocardiographic measurements in these patients.

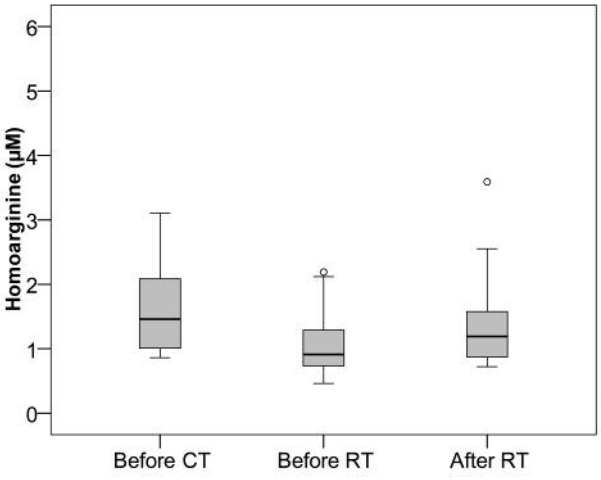

Figure 4. *The homoarginine (HA) level decreased during chemotherapy and increased during radiotherapy (RT) in the group treated with chemotherapy (CT).*

Discussion

Homoarginine mechanism. Homoarginine and ADMA are thought to be involved in the early process of atherosclerosis through their role in the regulation of nitric oxide (NO) production. An imbalance in NO and reactive oxygen species production, in turn, seems to lead to endothelial dysfunction, which contributes to the early process of atherosclerosis. Although the exact role of HA is unknown, it is assumed to lead to an increase in NO production through its role as a substrate of NO synthase and a substrate for arginase, which leads to increased arginine availability for NO synthase to produce NO (35). In animal models, tamoxifen caused NO-mediated vasodilatation (36-39) and reversed vascular dysfunction caused by ovariectomy (40). The NO-mediated effects may be responsible for the proposed cardioprotective effect of tamoxifen, attributed to its ability to lower low-density lipoprorein (LDL) and total cholesterol, cytokine-mediated anti-inflammatory effects and anti-oxidant properties, protecting LDL cholesterol from harmful oxidation (7).

It is also widely accepted that NO also plays a role in cardiotoxicity caused by chemotherapy, especially anthracyclines. Cardiotoxicity is partially mediated by anthracycline coupling with endothelial NO synthase, leading to formation of radical oxygen species (41). The

behavior of HA, supported by the echocardiographic findings shown in our study may be a reflection of these NO related events in the endothelium.

Homoarginine and adjuvant breast cancer treatments. Our study demonstrates HA behavior during adjuvant treatments of breast cancer. To our knowledge, there are no previous studies evaluating the effect of cancer therapies on the HA level. In chemotherapy-naïve patients, the HA level of tamoxifen users increased significantly, whereas that of AI users or those without endocrine therapy remained stable during RT. The baseline HA level of tamoxifen users was also higher than that of non users, most likely due to starting endocrine therapy prior to RT. A telephone query confirmed that most patients had started tamoxifen prior to RT. The trajectory model also supports the finding that tamoxifen increases the HA level; all tamoxifen users were in the two groups with the highest HA levels.

The HA levels in chemo-naïve statin users increased significantly, albeit slightly, during RT, whereas there was no significant change in HA in patients without statin use. Statin use was reported to have no effect on HA level (14) and its usefulness as a predictor of cardiovascular and all-cause mortality (11). As there were only 17 statin users, our findings could be due to chance, but the possible effects of statin use on HA level warrant further investigation as statins may have a role as cardioprotectant during chemotherapy (42).

Adjuvant chemotherapy also affected the HA level. As expected, due to adjuvant chemotherapy indications, patients who received chemotherapy were younger and more likely to receive tamoxifen or AI. During adjuvant RT, following chemotherapy, the HA level increased but did not reach starting levels in AI users, whereas in tamoxifen users, the HA level returned to that at baseline, indicating a cardioprotective effect of tamoxifen. The effect of statin use on HA level in chemotherapy-receiving patients was not determined, as there was only one statin-user receiving chemotherapy.

Other biomarkers. The level of ADMA did not change during adjuvant therapy. The ADMA level of patients receiving chemotherapy was lower than that of chemo-naïve patients, as ADMA tends to increase with age (43) and patients treated with chemotherapy were younger than chemo-naïve patients. ADMA appears to be insufficiently sensitive for detecting cardiovascular risk in our patients.

Earlier, we reported that RT induced an increase of hscTnT in chemo-naïve patients with left-sided breast cancer that was associated with cardiac radiation dose (21). In patients with chemotherapy, hscTnT increased during both chemotherapy and RT significantly. Some evidence of the possible cardioprotective effect of tamoxifen was also seen in hscTnT behavior, as during RT, further increase of hscTnT was only significant in AI users, not in tamoxifen users.

Table III. *Median (interquartile range) echocardiographic measurements and p-value for change from before and after radiotherapy (RT) in chemo-naive tamoxifen users, aromatase inhibitor (AI) users and patients without endocrine therapy.*

	Tamoxifen				AI				No endocrine therapy			
Median (IQR),	N	Before RT	After RT	*p*-Value	N	Before RT	After RT	*p*-Value	N	Before RT	After RT	*p*-Value
LVEDD mm	6	45.5 (43.6-48.0)	44.5 (43.6-48.0)	0.600	28	44.5 (41.6-47.0)	44.8 (41.6-46.7)	0.779	40	44.9 (42.5-47.5)	45.1 (41.3-47.5)	0.145
LVESD mm	6	31.4 (28.9-32.2)	30.6 (28.8-32.0)	0.917	28	30.3 (28.3-32.3)	30.2 (27.9-31.8)	0.453	40	30.4 (27.8-32.9)	30.7 (26.9-33.1)	0.521
IVS mm	6	9.5 (8.9-10.8)	10.1 (9.1-12.5)	0.116	28	10.4 (9.3-11.1)	10.4 (10.0-11.7)	0.160	41	10.0 (8.9-10.9)	10.0 (9.0-11.0)	0.023
PW mm	6	9.4 (9.0-12.1)	10.7 (10.0-11.6)	0.463	28	10.0 (9.6-11.0)	10.7 (9.9-11.9)	0.083	41	10.0 (9.0-10.4)	10.1 (9.0-10.9)	0.046
cIBS dB	6	−21.3 (−24.3–−16.4)	−14.6 (−19.4–−13.5)	0.345	25	−17.1 (−19.6–−13.3)	−16.6 (−18.9–−11.7)	0.211	36	−20.1 (−24.1–−15.6)	−15.9 (−21.3–−12.3)	0.002
LVEF %	5	67.0 (57.5-71.0)	66.0 (61.3-67.8)	0.892	26	68.0 (62.0-73.09	66.0 (58.0-69.0)	0.258	39	63.0 (60.0-66.0)	64.0 (61.0-69.0)	0.118
GLS %	6	−16.0 (−19.3–−14.8)	−18.0 (−21.3–−16.8)	0.078	25	−18.0 (−20.5–−15.0)	−17.0 (−20.0–−16.0)	0.001	37	−19.0 (−20.0–−16.0)	−16.5 (−19.0–−14.0)	0.679
CVIBS dB	6	12.9 (10.0-17.4)	8.9 (7.6-14.4)	0.046	25	9.9 (8.3-11.3)	9.5 (7.9-12.2)	0.667	35	11.8 (9.4-14.4)	9.3 (8.2-11.3)	0.001
Mitral E cm/s	6	81.0 (67.3-86.4)	77.1 (60.4-91.4)	0.917	28	76.5 (63.1-85.8)	67.6 (61.4-78.3)	0.003	41	70.1 (63.0-82.7)	66.7 (57.8-79.3)	0.448
Mitral Ee' ratio	6	8.8 (6.9-13.0)	7.5 (7.0-12.1)	0.753	28	10.2 (8.7-12.1)	9.4 (8.2-10.8)	0.246	41	8.3 (7.1-10.4)	8.4 (7.1-10.2)	0.591
TAPSE mm	5	25.0 (24.0-28.0)	25.0 (22.8-26.0)	0.461	28	24.0 (21.0-28.0)	21.5 (18.3-25.0)	<0.001	41	24.0 (20.5-27.5)	21.0 (19.5-25.5)	0.028
Tr grad mmHg	5	20.0 (17.0-25.5)	21.0 (17.8-24.3)	0.890	20	22.5 (19.0-25.3)	21.5 (19.3-24.0)	0.450	31	22.0 (17.0-25.0)	21.0 (17.5-23.5)	0.313

LVEDD/LVESD: Left ventricular end-diastolic/end-systolic diameter; IVS: interventricular septum; PW: posterior wall; cIBS: septal integrated backscatter in end-diastole calibrated to the pericardium; GLS: global longitudinal strain in speckle tracking analysis; CVIBS: septal cyclic variation of the integrated backscatter; Mitral E: mitral inflow E-wave peak velocity in pulsed Doppler analysis; Mitral Ee'-ratio: ratio between mitral inflow E-wave velocity and averaged pulsed tissue Doppler velocities derived from septal: lateral: anterior and posterior basal segments of the left ventricle; TAPSE: tricuspid annular plane systolic excursion; Tr grad: tricuspid regurgitation peak gradient; N: number of reliable paired measurements acquired.

Changes in echocardiographic measurements. RT-induced changes in echocardiographic measurements of our chemo-naïve patient population have been published previously (17, 21, 33, 34, 44). However, in this study, we reported the differences in echocardiographic changes in chemo-naïve patients according to endocrine therapy. The differences in baseline echocardiographic parameters according to endocrine therapy use are attributable to starting endocrine therapy before starting RT.

Patients using tamoxifen were spared most of the RT-induced changes. Only CVIBS, a sensitive marker of left ventricular systolic function, showed a significant decline with RT.

In AI users, there was a decline in TAPSE, a measurement of right ventricular systolic function, and GLS, a measurement of left ventricular myocardial performance, both prognostic markers in various heart diseases (25, 45-47). In addition, a decline in mitral E wave implies that diastology was also affected.

Patients without endocrine therapy exhibited structural changes in addition to functional changes, as the left ventricular wall thickened and its reflectivity increased after RT. As in our previous work, these changes might represent RT-induced tissue swelling as a result of early inflammatory changes (17, 24, 33, 34, 44). The functional changes were visible in ventricular function, namely, in TAPSE and CVIBS.

The changes during RT in echocardiographic parameters in chemo-naïve patients were similar to those we described previously (17, 24, 33, 34, 44). This study only included patients with measurable HA. Earlier, we reported a decrease in CVIBS induced by AI (24), but the study included patients excluded here due to missing serum samples. Furthermore, the laterality of breast cancer was not taken into consideration in our study. These facts might explain the different findings.

The echocardiographic findings of patients with chemotherapy in this population have not been previously published. Structural changes, namely, thickening of the ventricular wall, were seen during chemotherapy, as LVESD and PW increased. Functional changes were limited to a decline in the left ventricular systolic marker, CVIBS. A significant increase in Trgrad was also measured, but this cannot be considered clinically meaningful. RT, which was received after chemotherapy, did not induce any additional changes in echocardiography in these patients, but chemotherapy-induced thickening of LVESD persisted even after RT. Due to the small population that had both HA and echocardiographic measurements, we were not able to determine correlations between HA level and echocardiographic measurements.

Confounding factors and limitations. The small study population is a major limitation of our study, especially the small chemotherapy and tamoxifen-user groups. Therefore, we were not able to determine the effect of laterality of breast cancer on HA values, although the location of myocardial changes due to RT is dependent on the irradiated side. In the chemo-naïve patient population, tamoxifen and AI users had a higher BMI than patients who did not use endocrine therapy. A higher BMI has been associated with a higher HA level (9, 11, 12), which could have influenced our results. The concurrent use of endocrine therapy and RT leads to inability to separate the effects of these different treatments on echocardiographic parameters and biomarker measurements. Finally, the cardiovascular complications of adjuvant breast cancer treatments take years to manifest and therefore larger studies, with longer follow-up, are needed to confirm our results.

Clinical implications. Due to the excellent results of early breast cancer treatments, it is important to identify the patients at risk for cardiovascular morbidity and mortality. HA studies with longer follow-up could further elucidate the mechanisms behind chemotherapy-induced cardiotoxicity and the assumed cardioprotective effect of tamoxifen and provide an additional, available and minimally invasive tool to identify patients in need of cardiological follow-up.

Conflicts of Interest

None to declare.

Acknowledgements

Financial support was provided by the Seppo Nieminen fund and the competitive research funding of Tampere University Hospital.

References

1 Darby SC, Ewertz M, McGale P, Bennet AM, Blom-Goldman U, Bronnum D, Correa C, Cutter D, Gagliardi G, Gigante B, Jensen MB, Nisbet A, Peto R, Rahimi K, Taylor C and Hall P: Risk of ischemic heart disease in women after radiotherapy for breast cancer. N Engl J Med *368*: 987-998, 2013.

2 Ades F, Zardavas D, Pinto AC, Criscitiello C, Aftimos P and de Azambuja E: Cardiotoxicity of systemic agents used in breast cancer. Breast *23*: 317-328, 2014.

3 Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Darby S, McGale P, Correa C, Taylor C, Arriagada R, Clarke M, Cutter D, Davies C, Ewertz M, Godwin J, Gray R, Pierce L, Whelan T, Wang Y and Peto R: Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet *378*: 1707-1716, 2011.

4 Bardia A, Arieas ET, Zhang Z, Defilippis A, Tarpinian K, Jeter S, Nguyen A, Henry NL, Flockhart DA, Hayes DF, Hayden J, Storniolo AM, Armstrong DK, Davidson NE, Fetting J, Ouyang P, Wolff AC, Blumenthal RS, Ashen MD and Stearns V: Comparison of breast cancer recurrence risk and cardiovascular disease incidence risk among postmenopausal women with breast cancer. Breast Cancer Res Treat *131*: 907-914, 2012.

5 Bouillon K, Haddy N, Delaloge S, Garbay JR, Garsi JP, Brindel P, Mousannif A, Le MG, Labbe M, Arriagada R, Jougla E, Chavaudra J, Diallo I, Rubino C and de Vathaire F: Long-term cardiovascular mortality after radiotherapy for breast cancer. J Am Coll Cardiol *57*: 445-452, 2011.

6 van den Bogaard VA, Ta BD, van der Schaaf A, Bouma AB, Middag AM, Bantema-Joppe EJ, van Dijk LV, van Dijk-Peters FB, Marteijn LA, de Bock GH, Burgerhof JG, Gietema JA, Langendijk JA, Maduro JH and Crijns AP: Validation and modification of a prediction model for acute cardiac events in patients with breast cancer treated with radiotherapy based on three-dimensional dose distributions to cardiac substructures. J Clin Oncol *35*: 1171-1178, 2017.

7 Khosrow-Khavar F, Filion KB, Al-Qurashi S, Torabi N, Bouganim N, Suissa S and Azoulay L: Cardiotoxicity of aromatase inhibitors and tamoxifen in post-menopausal women with breast cancer: a systematic review and meta-analysis of randomized controlled trials. Ann Oncol *28*: 487-496, 2016.

8 Pilz S, Meinitzer A, Tomaschitz A, Drechsler C, Ritz E, Krane V, Wanner C, Boehm BO and Marz W: Low homoarginine concentration is a novel risk factor for heart disease. Heart *97*: 1222-1227, 2011.

9 Atzler D, Gore MO, Ayers CR, Choe CU, Boger RH, de Lemos JA, McGuire DK and Schwedhelm E: Homoarginine and cardiovascular outcome in the population-based Dallas Heart Study. Arterioscler Thromb Vasc Biol *34*: 2501-2507, 2014.

10 Atzler D, Baum C, Ojeda F, Keller T, Cordts K, Schnabel RB, Choe CU, Lackner KJ, Munzel T, Boger RH, Blankenberg S, Schwedhelm E and Zeller T: Low homoarginine levels in the prognosis of patients with acute chest pain. J Am Heart Assoc *5*: e002565, 2016.

11 Marz W, Meinitzer A, Drechsler C, Pilz S, Krane V, Kleber ME, Fischer J, Winkelmann BR, Bohm BO, Ritz E and Wanner C: Homoarginine, cardiovascular risk, and mortality. Circulation *122*: 967-975, 2010.

12 Pilz S, Teerlink T, Scheffer PG, Meinitzer A, Rutters F, Tomaschitz A, Drechsler C, Kienreich K, Nijpels G, Stehouwer CD, Marz W and Dekker JM: Homoarginine and mortality in an older population: the Hoorn study. Eur J Clin Invest *44*: 200-208, 2014.

13 Atzler D, Rosenberg M, Anderssohn M, Choe CU, Lutz M, Zugck C, Boger RH, Frey N and Schwedhelm E: Homoarginine – an independent marker of mortality in heart failure. Int J Cardiol *168*: 4907-4909, 2013.

14 Tomaschitz A, Meinitzer A, Pilz S, Rus-Machan J, Genser B, Drechsler C, Grammer T, Krane V, Ritz E, Kleber ME, Pieske B, Kraigher-Krainer E, Fahrleitner-Pammer A, Wanner C, Boehm BO and Marz W: Homoarginine, kidney function and cardiovascular mortality risk. Nephrol Dial Transplant *29*: 663-671, 2014.

15 Pilz S, Edelmann F, Meinitzer A, Gelbrich G, Doner U, Dungen HD, Tomaschitz A, Kienreich K, Gaksch M, Duvinage A, Stahrenberg R, Kunde J, Schmidt A, Marz W, Wachter R and Pieske B: Associations of methylarginines and homoarginine with diastolic dysfunction and cardiovascular risk factors in patients with preserved left ventricular ejection fraction. J Card Fail *20*: 923-930, 2014.

16 Willeit P, Freitag DF, Laukkanen JA, Chowdhury S, Gobin R, Mayr M, Di Angelantonio E and Chowdhury R: Asymmetric dimethylarginine and cardiovascular risk: systematic review and meta-analysis of 22 prospective studies. J Am Heart Assoc *4*: e001833, 2015.

17 Skytta T, Tuohinen S, Virtanen V, Raatikainen P and Kellokumpu-Lehtinen PL: The concurrent use of aromatase inhibitors and radiotherapy induces echocardiographic changes in patients with breast cancer. Anticancer Res *35*: 1559-1566, 2015.

18 Joensuu H, Kellokumpu-Lehtinen PL, Huovinen R, Jukkola-Vuorinen A, Tanner M, Asola R, Kokko R, Ahlgren J, Auvinen P, Hemminki A, Paija O, Helle L, Nuortio L, Villman K, Nilsson G, Lahtela SL, Lehtio K, Pajunen M, Poikonen P, Nyandoto P, Kataja V, Bono P, Leinonen M, Lindman H and FinXX Study Investigators: Adjuvant capecitabine in combination with docetaxel and cyclophosphamide plus epirubicin for breast cancer: an open-label, randomised controlled trial. Lancet Oncol *10*: 1145-1151, 2009.

19 Joensuu H, Kellokumpu-Lehtinen PL, Bono P, Alanko T, Kataja V, Asola R, Utriainen T, Kokko R, Hemminki A, Tarkkanen M, Turpeenniemi-Hujanen T, Jyrkkio S, Flander M, Helle L, Ingalsuo S, Johansson K, Jaaskelainen AS, Pajunen M, Rauhala M, Kaleva-Kerola J, Salminen T, Leinonen M, Elomaa I, Isola J and FinHer Study Investigators: Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. N Engl J Med *354*: 809-820, 2006.

20 Joensuu H, Bono P, Kataja V, Alanko T, Kokko R, Asola R, Utriainen T, Turpeenniemi-Hujanen T, Jyrkkio S, Moykkynen K, Helle L, Ingalsuo S, Pajunen M, Huusko M, Salminen T, Auvinen P, Leinonen H, Leinonen M, Isola J and Kellokumpu-Lehtinen PL: Fluorouracil, epirubicin, and cyclophosphamide with either docetaxel or vinorelbine, with or without trastuzumab, as adjuvant treatments of breast cancer: final results of the FinHer Trial. J Clin Oncol *27*: 5685-5692, 2009.

21 Skytta T, Tuohinen S, Boman E, Virtanen V, Raatikainen P and Kellokumpu-Lehtinen PL: Troponin T-release associates with cardiac radiation doses during adjuvant left-sided breast cancer radiotherapy. Radiat Oncol *10*: 141, 2015.

22 Teerlink T, Nijveldt RJ, de Jong S and van Leeuwen PA: Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. Anal Biochem *303*: 131-137, 2002.

23 Valtonen P, Karppi J, Nyyssonen K, Valkonen VP, Halonen T and Punnonen K: Comparison of HPLC method and commercial ELISA assay for asymmetric dimethylarginine (ADMA) determination in human serum. J Chromatogr B Analyt Technol Biomed Life Sci *828*: 97-102, 2005.

24 Tuohinen SS, Skytta T, Huhtala H, Virtanen V, Virtanen M, Kellokumpu-Lehtinen PL and Raatikainen P: Detection of early radiotherapy-induced changes in intrinsic myocardial contractility by ultrasound tissue characterization in patients with early-stage breast cancer. Echocardiography *34*: 191-198, 2017.

25 Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W and Voigt JU: Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging *16*: 233-270, 2015.

26 Galderisi M, Henein MY, D'hooge J, Sicari R, Badano LP, Zamorano JL, Roelandt JR and European Association of Echocardiography: Recommendations of the European Association of Echocardiography: How to use echo-Doppler in clinical trials: different modalities for different purposes. Eur J Echocardiogr *12*: 339-353, 2011.

27 Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA and Evangelisa A: Recommendations for the evaluation of left ventricular diastolic function by echocardiography. Eur J Echocardiogr *10*: 165-193, 2009.

28 Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, Solomon SD, Louie EK and Schiller NB: Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. J Am Soc Echocardiogr *23*: 685-713, 2010.

29 Nagin D: Group-Based Modeling of Development. Cambridge, MA, Harvard Univesity Press, 2005.

30 Nagin DS and Odgers CL: Group-based trajectory modeling in clinical research. Annu Rev Clin Psychol *6*: 109-138, 2010.

31 Leisch F: A general framework for finite mixture models and latent class regression in R. J Stat Soft *11*: 1-18, 2004.

32 R Development Core Team (2016): R: A language and environment for statistical computing, Vienna, Austria. http://www.R-project.org/

33 Tuohinen SS, Skytta T, Virtanen V, Virtanen M, Luukkaala T, Kellokumpu-Lehtinen PL and Raatikainen P: Detection of radiotherapy-induced myocardial changes by ultrasound tissue characterisation in patients with breast cancer. Int J Cardiovasc Imaging *32*: 767-776, 2016.

34 Tuohinen SS, Skytta T, Poutanen T, Huhtala H, Virtanen V, Kellokumpu-Lehtinen PL and Raatikainen P: Radiotherapy-induced global and regional differences in early-stage left-sided versus right-sided breast cancer patients: speckle tracking echocardiography study. Int J Cardiovasc Imaging *33*: 463-472, 2016.

35 Papageorgiou N, Androulakis E, Papaioannou S, Antoniades C and Tousoulis D: Homoarginine in the shadow of asymmetric dimethylarginine: from nitric oxide to cardiovascular disease. Amino Acids *47*: 1741-1750, 2015.

36 Figtree GA, Webb CM and Collins P: Tamoxifen acutely relaxes coronary arteries by an endothelium-, nitric oxide-, and estrogen receptor-dependent mechanism. J Pharmacol Exp Ther 295: 519-523, 2000.

37 Leung HS, Yung LM, Leung FP, Yao X, Chen ZY, Ko WH, Laher I and Huang Y: Tamoxifen dilates porcine coronary arteries: roles for nitric oxide and ouabain-sensitive mechanisms. Br J Pharmacol *149*: 703-711, 2006.

38 Montenegro MF, Ceron CS, Salgado MC, Desta Z, Flockhart DA and Tanus-Santos JE: Tamoxifen and its metabolites cause acute vasorelaxation of aortic rings by inducing vasodilator prostanoid synthesis. J Cardiovasc Pharmacol *58*: 647-653, 2011.

39 Borgo MV, Lopes AB, Gouvea SA, Romero WG, Moyses MR, Bissoli NS and Abreu GR: Effect of tamoxifen on the coronary vascular reactivity of spontaneously hypertensive female rats. Braz J Med Biol Res *44*: 786-792, 2011.

40 Lamas AZ, Caliman IF, Dalpiaz PL, de Melo AF,Jr, Abreu GR, Lemos EM, Gouvea SA and Bissoli NS: Comparative effects of estrogen, raloxifene and tamoxifen on endothelial dysfunction, inflammatory markers and oxidative stress in ovariectomized rats. Life Sci *124*: 101-109, 2015.

41 Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ and Moens AL: Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J Mol Cell Cardiol *52*: 1213-1225, 2012.

42 Seicean S, Seicean A, Plana JC, Budd GT and Marwick TH: Effect of statin therapy on the risk for incident heart failure in patients with breast cancer receiving anthracycline chemotherapy: an observational clinical cohort study. J Am Coll Cardiol *60*: 2384-2390, 2012.

43 Atzler D, Schwedhelm E, Nauck M, Ittermann T, Boger RH and Friedrich N: Serum reference intervals of homoarginine, ADMA, and SDMA in the study of health in Pomerania. Clin Chem Lab Med *52*: 1835-1842, 2014.

44 Tuohinen SS, Skytta T, Virtanen V, Luukkaala T, Kellokumpu-Lehtinen PL and Raatikainen P: Early effects of adjuvant breast cancer radiotherapy on right ventricular systolic and diastolic function. Anticancer Res *35*: 2141-2147, 2015.

45 Damy T, Kallvikbacka-Bennett A, Goode K, Khaleva O, Lewinter C, Hobkirk J, Nikitin NP, Dubois-Rande JL, Hittinger L, Clark AL and Cleland JG: Prevalence of, associations with, and prognostic value of tricuspid annular plane systolic excursion (TAPSE) among out-patients referred for the evaluation of heart failure. J Card Fail *18*: 216-225, 2012.

46 Leong DP, Hoke U, Delgado V, Auger D, Witkowski T, Thijssen J, van Erven L, Bax JJ, Schalij MJ and Marsan NA: Right ventricular function and survival following cardiac resynchronisation therapy. Heart *99*: 722-728, 2013.

47 Alhamshari YS, Alnabelsi T, Mulki R, Cepeda-Valery B, Figueredo VM and Romero-Corral A: Right ventricular function measured by TAPSE in obese subjects at the time of acute myocardial infarction and 2year outcomes. Int J Cardiol *232*: 181-185, 2017.

Received September 22, 2017
Revised October 15, 2017
Accepted October 19, 2017